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EFFECT OF HEATING AND STORAGE TIME LEVELS 5- (Hydroxy Methyl) furan-2- KARBALDEHIDA (HMF) IN HONEY ORIGIN MALLAWA

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ABSTRACT

A research has been on determining the origin of forest honey quality Mallawa Maros based on the levels of 5- (hydroxymethyl) furfural-2-karbaldehida (HMF) is quantitatively based on the variation of heating temperature and duration of storage with White spectrophotometric method. The results showed that the levels of forest honey HMF origin Mallawa on heating temperature 35 ° C, 90 ° C and 110 ° C respectively is 42.2023 mg / Kg, 46.0274 mg / Kg and 62.2520 mg / Kg. While the storage duration for 3, 4, and 5 months had higher levels of HMF each 27.4309 mg / Kg, 42.5158 mg / Kg and 48.9405 mg / Kg. Heating temperature and storage time a big influence on levels of HMF and show a strong relationship to the water content, pH and free acid, reducing sugar content and diastase enzyme activity. Based on the research results, levels of HMF at the heating temperature of 110 ^{o}C has passed the standards set by the SNI (up to 50 mg / Kg). Whereas the standard set by IHC for honey originating from the tropics (80 mg / Kg).

Keywords: HMF, Honey, Mallawa, storage, chemicalproperties, temperature.

1. INTRODUCTION

According to the Indonesian National Standard (SNI) 01-3545-2004, honey is a sweet liquid produced by honeybees from various sources of nectar. Honey is a kind of liquid produced by the glands of nectar plants, rich in a variety of carbohydrate (3-87%), such as sucrose, fructose and glucose, containing a small amino acid compounds, amides, organic acids, vitamins, aromatic compounds and minerals. Honey contains fructose 41.0%, 35.0% glucose, sukrosa1.9%, dextrin 1.5%, l0.2% minerals, 17% water and other substances such as amino acids 3.5%.[7]

Provinces in Sulawesi who are developing as a leading commodity honey timber forest products is southern Sulawesi with a program of Community Economic Development Movement, known as the Golden Gate. This activity is held development programs beekeeping as poverty reduction activities are carried out in several areas in South Sulawesi as Gowa, Bantaeng, Sinjai, Bulukumba, Maros, Sidrap, Palopo, Tana Toraja, Luwu, Bone, and several other areas.[11] Maros is the district center and incubator development of honey bees in South Sulawesi.[11] Administratively, the district Marosterbagi into 14 districts. One of the districts listed in Maros is Mallawa who finished second largest with an area of 287.66km2. In the market plenty of forgery honey could reduce the quality of honey. Fake honey made without the help of bees or use sugar as nectar. Generally have the same colors as the original honey. Therefore, for the layman is difficult to differentiate between

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honey and honey imitation. All forms of counterfeiting of honey begins on consumer demand for honey with a very cheap price. By it necessary quantitative testing to ensure the authenticity of honey. The real difference between pure honey and pure honey does not lie in their chemical composition.[13] The chemical composition of honey which can be an indicator of the purity of honey that is the content of HMF (5-hydroxymethyl-2-furfural), moisture content, carbohydrate, protein, and the pH value.[5]

HMF (5-hydroxymethyl-2-furfural) is one of the parameters of honey damage. HMF is a decomposition product of sugars formed in honey during heat processing and storage, otherwise known as the Maillard reaction.[14] Testing levels of HMF in honey is very important in determining the authenticity and freshness of the honey. Factors affecting the increase in the levels of HMF in honey are temperature, length of storage and the addition of fructose. The rise in temperature can increase levels of HMF, this is evidenced by several studies stating that at a temperature of 4-65 °C concentration HMF in honey is still below 40 mg/kg.[15],[2] and Sujirapinyokul 2010; Esceriche et al., 2008) but when the honey is stored at a temperature of 70 °C after 96 hours the levels of HMF in honey at 91 mg / kg (Fellico et al., 2004). Time is also an important factor in the formation of HMF. Honey samples were stored for 4 years at a temperature of 20 °C, can increase levels of HMF as much as 52.44%.[10] Moreover, the addition of fructose as a sweetener can also increase the levels of HMF up to 100 mg / kg.[12] The maximum levels of 5-hydroxymethyl-2-furfural (HMF) in honey established by the Codex Alimentarius is less than 60 mg / kg. European Union determine maximum limit of 5-hydroxymethyl-2-furfural (HMF) in honey which is 40 mg / kg except for the honey that comes from tropical climates that is no greater than 80 mg / kg.[6] SNI set the levels of 5-hydroxymethyl-2-furfural (HMF) in honey is not allowed to exceed 50 mg / kg (SNI number 3545: 2013).

The main problems are often found in honey Indonesia is a high water content compared with honey-honey from other countries. This is due to the tropical climate of Indonesia with high rainfall directly affects the honey produced. Honey with water content of 18.3% or less than that, it will absorb moisture from the air at relative humidity above 60%. Honey is a very hygroscopic material, so that in a humid climate to be high water content compared to the dry climate.[6]

During the preparation and handling, honey should only be heated minimal probably because of excessive heating can increase levels of HMF and lowers the activity of the enzyme diastase thus decreasing the quality of honey. Even if the liveliness of diastase to 0 and the levels of HMF>40 mg/kg possibility honey has been adulterated with invert sugar. The quality of honey from South Sulawesi Maros particularly Mallawa has never been demonstrated that there is no data to support the quality of the honey. Thus, necessary to test the quality of honey quantitative basis by analyzing the levels of 5-hydroxymethyl-2-furfural contained in the honey origin Mallawa South Sulawesi

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2. MATERIALS AND METHODS

Materials Research

The materials used in this study is the origin of forest honey Mallawa, Potassium hexacyanoferrate (K4Fe (CN) 6.3H2O), zinc acetate (Zn (CH3COO) 2, sodium bisulfite (NaHSO3) 0.20%, iodine stock solution, buffer solution acetate, sodium chloride (NaCl) 0.5 M, solution of nitric acid (HNO3) 5 N, 42 Whatman filter paper, Sodium hydroxide (NaOH) 0.5 N, deionized water, hydrochloric acid (HCl) pa 37%, distilled water, acid 3,5-dinitrosalisilat (DNS), the indicator PP, KNA-Tartrate 40%.(HNO3) 5 N, 42 Whatman filter paper, Sodium hydroxide (NaOH) 0.5 N, deionized water, hydrochloric acid (HCl) pa 37%, distilled water, acid 3,5-dinitrosalisilat (DNS), the indicator PP, KNA-Tartrate 40%.

Research Time And Place Sampling

This study was conducted in April 2015 to completion in the Radiation Chemistry Laboratory Chemistry Department, Faculty of Mathematics and Natural Sciences University of Hasanuddin. While sampling was conducted in the forest honey Tellupanuae Village, District Mallawa, Maros South Sulawesi.

Preparation Of Sample

Storage Time Treatment

Samples were inserted into the vial 3 bottles each of 50 mL. After that, each labeled storage of 3 months, 4 months and 5 months. Then stored at room temperature (28 ^{o}C).

Heating Treatment

Samples of honey put into 3 pieces each beaker 25 mL. then heated in an oven at various temperatures. Beaker 1 is heated at a temperature of 35 ° C, beaker 2 at 90 ^{o}C and beaker 3 at 110 ^{o}C. each heated for 1 hour and then cooled in a desiccator.

Research Procedure

Determination of Levels of 5- (hydroxy methyl) furan-2-karbaldehid (HMF)

Samples of honey each weighed as much as approximately 5 grams and diluted with distilled water as much as 25 mL and then put in a 50 mL volumetric flask. After that, the solution was added with 0.5 mL of Carrez I then whipped and then added with a solution of 0.5 ml Carrez II and shaken back. The solution is then diluted with distilled water up to the mark and then added with a few drops of methanol. Then filtered through a filter paper, and 10 mL of the first filtrate discarded. Subsequently pipette 5 mL filtrate and put into two different test tubes. In the first test tube was added with 5 mL of distilled water while in the second test tube was added with 5 mL of sodium bisulfit0,2% as a comparison. Both test tubes each shaken until completely mixed. Furthermore, an absorbance of the sample was measured, the first test tube was measured at a wavelength of 284 nm, while the second test tube was

Analysis Of Chemical Characteristics Of Honey

Characteristic chemical origin honey Mallawa analyzed to determine the effect on honey HMF content in the form of a water content, pH and acidity, reducing sugar content and origin of honey diastase enzyme activity.

Determination of the water content of honey was conducted using a refractometer the refractive index of honey

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with readings conducted at a temperature of 20 ^{o}C. Then the water levels are compared with the value of the refractive index and water content in the table determination of water content by the International Honey Commission (2002). pH and acid-free acid-free and pH levels were determined by Lutron pH-meter base on International Honey Commission (2002). Reducing sugar content determined using the method of Luff- Schrool calculate percent sugar before inversion. Diastase enzyme activity was determined using the schade namely by calculating mL 1% starch hydrolyzed for one hour at a temperature of 40 ^{0}C measured at a wavelength of 336 nm using a UV-Vis spectrophotometer (Shimadzu UV-2600).

RESULT AND DISCUSSION

The results of the analysis on the chemical properties of honey origin Mallawa based heating temperature variations such as the determination of water content, acidity and pH, reducing sugars, enzymes diastase and HMF, can be seen in Table 1, while for storage time variations can be seen in Table 2.

Table 1. Chemical Properties of Honey Origin Mallawa Based Temperature Variations heating

Heating honey (^{o}C)	Water Content (% w /w)	Acidity (meq /kg)	pH	Sugar Reduction (%)	Enzyme Diastase (DN)	HMF (mg/Kg)
35 ^{o}C	23,00	34,38	4,31	69,96	10.48	42.20
90 ^{o}C	14,70	43,48	4,22	66,61	6.66	46.03
110 ^{o}C	12,30	48,76	4,12	65,60	2.50	62.25

Table 2. Chemical Properties of Honey Origin Mallawa Based on Time Variation Storage

Storage honey (month)	Water Content (% w /w)	Acidity (meq /kg)	pH	Sugar Reduction (%)	Enzyme Diastase (DN)	HMF (mg/Kg)
3 months	22.40	30.53	4.48	67.25	11.30	27.43
4 months	22.60	43.10	4.47	65.21	9.212	42.52
5 months	23.20	47.90	4.38	63.96	5.42	48.94

Determination of Levels of 5- (hydroxy methyl) furan-2-karbaldehid (HMF)

Based on Table 1, it can be seen that the temperature greatly affects the levels of HMF. The content of HMF in honey is a chemical compound that is produced from honey overhaul monosaccharides (glucose and fructose) under acidic conditions with the help of heat (heat).[1] At the highest levels of HMF heating temperature of 110 ^{o}C HMF levels reached 62.25 mg / 100g, whereas at 90 ° C and 35 ° C respectively (46.03 mg / 100gdan 42.20

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mg / 100g). It shows that the higher the heating temperature can increase the speed of reaction of formation of HMF in honey Mallawa origin. It corresponds to the white (1979) which states that in addition to acid catalyzed, formation of HMF was also catalyzed by the temperature.

The results of the analysis of the levels of HMF honey origin Mallawa showed that heating at a temperature of 110° C, the content of HMF has passed the standards set by the ISO (<50 mg / kg) but still below the standards set by IHC for honey originating from tropical regions namely (<80 mg / kg). While in heating 35 °C and 90 °C respectively under ISO standard and IHC.

Furthermore, in Table 2 for the treatment of storage time showed that, on storage for 5 months had higher levels of HMF highest of 48.94 mg / 100g, and storage levels of HMF in 4 months and 3 months respectively (42.52 mg / and 27,43mg 100g / 100g). Based on that data, HMF levels will increase along with the storage time. This is in accordance Achmadi (1991) which states that in addition to the heating temperature, levels of HMF was also influenced by storage time, it relates to the Maillard reaction that occurs continuously during the storage period.

Water Content

Analysis of the water content of honey origin Mallawa in Table 1 show that, at 35 ° C warming containing a high water content which is 23.00% which has passed the standards set by SNI and IHC while heating at a temperature of 90 ° C and 110 ° C has a moisture content low and below the ISO standard and IHC, respectively 14.70% and 12.30%. From the analysis it can be seen that a decrease in the water content of honey can be done with heating. But lowering the water content in honey by warming is not the right way because it will be increased levels of HMF. Can be seen in Table 1, although the water content of honey after heating will decrease but HMF content will only increase. The content of HMF is high during the heating 110 ^{0}C and 90 ^{0}C which was respectively 62.25 mg / kg and 46.03 mg / kg and the lowest at 35 ^{0}C heating temperature that is 42.20 mg / Kg. This happens because when the water content in honey is heated to evaporate and the reaction of HMF formation will be faster. Therefore, the heating should be done in a controlled manner with a relatively short time.

Furthermore, the treatment duration of storage can be seen in Table 2, which shows that the highest water levels in the storage of 5 months (23.20%), then on the storage of 4 months (22.60%) and the lowest is in storage for 3 months (22 , 40%). The data indicate that the origin Mallawa honey has a high water content and passes ISO standard and IHC. This shows that the water content in honey has increased along with storage time. High levels of honey water Mallawa origin during storage is caused by several factors Mallawa tropical climate with high humidity to the hygroscopic properties of honey so that the honey will be easier to absorb water, and occurs naturally as the harvesting process is done during the rainy season is February, 2015.

Determination Of Free Acid And pH

Based on the data in Table 1, the results of the analysis of the free acid

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content in honey origin Mallawa based on variations in the heating temperature indicates that, of the free acid which is highest in the heating 110oC (48.76 mEq / Kg), then at a temperature of 90 °C the value of acidity (43,48meq / Kg) and the lowest is at 35 °C (34,38meq / Kg). The results show that the acidity of honey origin Mallawa still lower than the standard SNI however, to the standards set by IHC only honey on a warm 35 °C which meet the standard. Acid is a substance soluble in water and producing hydrogen and an organic acid weak that capable of releasing H^+ ions when experiencing high temperature heating (Winarno et al., 1984) .According to Winarno (1992), the causative agent of sour taste is the H^+ ions, if concentration hydrogen (acidity) increases the pH will drop and HMF content will be higher. This was caused by an increase in the heating temperature causes some water content in honey undergo evaporation and the faster the rate of formation of HMF.

Furthermore, for the treatment of storage duration has shown that honey is stored for 5 months to have a value higher acidity that is (47.896 mEq / kg) compared with the storage time 4 months and 3 months respectively (30.534 meq / Kg and 43,100 meq / Kg). This shows that during the period of storage of the free acid content in honey continues to increase and stimulate fermentation by bacteria and microbes that ferment lain.Hasil will form ethyl alcohol and carbon dioxide.

Determination of Reducing Sugar

Based on the data in the table above, three samples of honey have passed the minimum standards set by ISO and IHC. This shows that the higher the sugar content pereduksinya warming will decrease. This is caused by the sucrose content of the honey will also be inverted due to heating. Sucrose is a non-reducing because it has no free OH groups are reactive, but during heating in the presence of acid, sucrose to be hydrolyzed into sugars invert ie fructose and glucose is a reducing sugar, but at high temperatures of 90 and 110 decomposition of glucose and fructose to HMF will faster thus reducing sugar levels will decrease and the amount of HMF are accumulating more and more.

In addition, these data indicate that the storage time reduce the content of reducing sugars. It is influenced by the activity of enzymes that hydrolyze sucrose into glucose and fructose, but if too long a reducing sugar content in honey decreased with increased levels of HMF is a decomposition of simple sugars in honey during stockpiles.

Determination of Enzyme Activity Diastase

Heating temperature of 35 °C enzyme activity diastasenya (10,4823DN), at a temperature of 90 ^oC (6,6578DN) and at a temperature of 110 oC (2,4997DN). Based on SNI honey has at least diastase enzyme activity (min 3 DN) while IHC sets (min 8 DN). The data show that on heating 35 ° C and 90 ° C is still above the standard set by SNI. Whereas with IHC only honey in warm 35 ° C which is still above the standard. It shows that heating at temperatures higher can reduce the activity of the enzyme diastase. Changes in temperature will affect the

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activity of the enzyme, where at low temperatures the enzyme reaction rate will slow moving with kinetic energy and the impact of low molecular so as not to reach the activation energy sufficient for reaction.

Furthermore, the storage duration of treatment showed that honey that has been stored for 5 months of enzyme activity diastase most low at (5.4230 DN), then for months to 4 (9.2084 DN) and at months 3 enzyme activity diastasenya most high (11.2972 DN). This shows that the longer storage diastase enzyme activity will decline, besides the formation reaction of reducing sugars will decrease will tetapireaksi HMF formation derived from the decomposition of reducing existing gulua in honey will get faster so HMF levels will increase

CONCLUSIONS

1. Based on research that has been done, it can be concluded that: Levels of HMF in honey samples Mallawa origin Maros using spectrophotometric method for variation of the old White submarine storage of 3 months, 4 months and 5 months respectively obtained was 27.4309 mg / kg; 42.5158 mg / kg; 48.9405 mg / kg. As for the heating temperature variations, at a temperature of 35 0C, 90 0C and 110 0C have levels of HMF each of 42.2023 mg / kg; 46.0274 mg / kg; and 62.2520 mg / kg.
2. The levels of HMF increased during the heating process, along with a decrease in water content, reducing sugar, diastase enzyme activity and increased levels of free acid. As for the duration of storage, HMF levels will increase, along with an increase in moisture content, acid-free, and a decrease in reducing sugars and diastase enzyme activity.

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